Kinetics of Superoxide-Mediated Reduction of Organically Complexed Iron and Ferric Iron Oxyhydroxide in Coastal Waters

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論文内容要旨

1. Introduction and Objectives

Iron is widely recognized as one of the most essential micronutrients for almost all aquatic microorganisms. Over the last several decades, there has been intense interest in the biogeochemistry of iron with particular attention given to better understanding the thermodynamics of the Fe(II) and Fe(III) states, the kinetics of transformation between Fe(II) and Fe(III) species and the biological availability of these species. Although the speciation of iron is influenced by a variety of factors, the ferric iron state is generally considered to be the dominant form in surface coastal waters. Speciation of ferric iron in natural water can be mainly classified into two forms; organically complexed ferric iron (Fe(III)L) and amorphous ferric oxyhydroxide (AFO). However, both forms of iron are largely membrane impermeable due to the complicated and variable structure, such that it is unlikely that they are directly internalized by microorganisms. The reduction of ferric iron species by reductant such as superoxide anion radical and extracellular reductase increases the bioavailability of iron both, because ferrous iron is substantially more soluble than ferric iron and because any organic complexes that may form are substantially weaker than their ferric counterparts (Figure 1). Understanding of the key processes accounting for the reduction of ferric iron in aquatic environments is vital therefore for the understanding of biological acquisition of iron in seawater. The aim of this thesis is to kinetically investigate the mechanism of superoxide-mediated iron transformation in coastal waters.

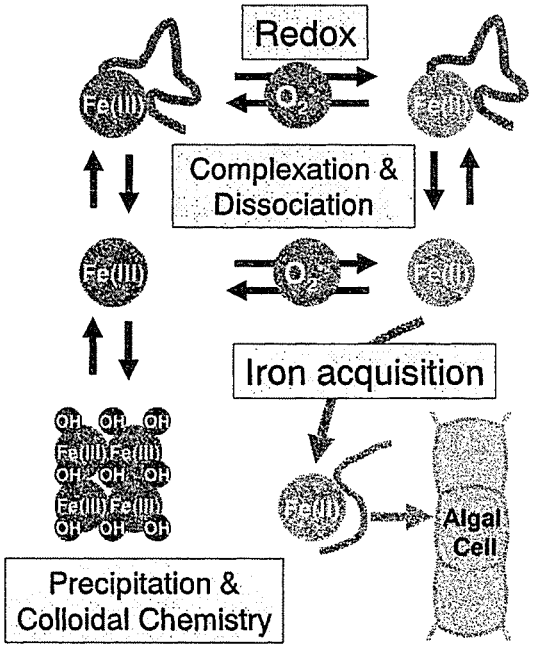

Figure 1 Iron species and speroxide-mediated iron uptake by microorganisms in coastal waters

2. Superoxide-mediated dissolution of amorphous ferric oxyhydroxide in seawater

The SO-mediated dissolution of AFO was firstly investigated by spectrophotometrically examining the rate of formation of a ferrous-ferrozine complex ($Fe(II)(FZ)_3$) with particular attention given to the effect of ageing and iron concentration on the rate of SO-mediated dissolution of AFO (Figure 2). It was found that superoxide generated at rates typical of those expected in natural aquatic systems is capable of reducing Fe(III) present in amorphous ferric oxide. The first order rate constants of overall reduction of Fe(III) (k'_{red}) are strongly dependent upon the age of the AFO, with a decrease in k'red by approximately an order of magnitude on ageing of AFO for one week. The results of our studies indicate that thermal dissociation (dissolution) of AFO occurs prior to reduction of dissolved inorganic iron by superoxide. Whether such a reductive process influences the bioavailability of iron to microorganisms will most likely be dependent to a large extent on the mechanism by which the organism acquires its iron supply. If the organism has an affinity for reduced iron species, any Fe(II)' produced as a result of the superoxide-mediated reduction of Fe(III) may be rapidly bound by strong ferrous binding ligands at the cell surface and subsequently internalized. Alternatively, if strong Fe(II)-binding ligands are not present to trap the ferrous iron, it is likely be rapidly oxidized back to the ferric state either in bulk solution or at the cell surface, especially if strong Fe(III) binding ligands are present.

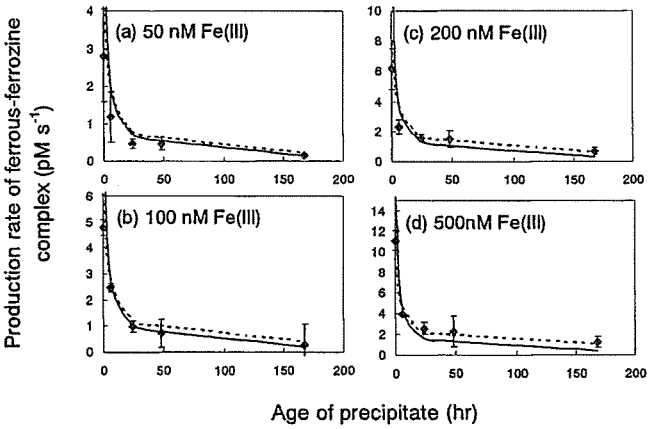

Figure 2. Production rates of ferrous-ferrozine complex from superoxide-mediated dissolution of amorphous ferric oxyhydroxide in seawater (pH 8.2), with addition of (a) 50 nM Fe(III), (b) 100 nM Fe(III), (c) 200 nM Fe(III) and (d) 500 nM Fe(III). The solid line is the line of model fit and the dashed line is the best fit using the modified model.

3. Development of an ion exchange separation method for characterization of the form and reactivity of iron in coastal waters

In this work, a novel technique for the selective separation of iron complexed by organic ligands and ligand-adsorbed AFO by using AER has been developed (Figure 3) and its viability for characterisation of the form of ferric iron in seawater was confirmed through laboratory-based experiments on seawater containing various mixtures of precipitated and organically complexed Fe(III). AFO aggregates associated with organic ligands undergo acid-mediated dissolution relatively slowly and appear to maintain residual negative charge at low pH; thus they are relatively easily separated by the AER treatment from dissolved Fe(III) organic complexes, which dissociate rapidly to form positively charged ferric species.

In addition to confirming the viability of the acidifircation/AER method, the results reported here also provide insight to the reactivity of the various Fe(III) species present in seawater. In particular, the results confirm the dramatic effect of ageing on AFO reactivity and indicate that the presence of organics on the AFO surface greatly reduces the reactivity of these Fe(III) particulates. Additionally, it appears that the kinetics of transformation of dissolved Fe(III) organic complexes may be mediated by association of these complexes with iron oxyhydroxide particulates. These factors are of importance to the bioavailability of iron to organisms and require further investigation.

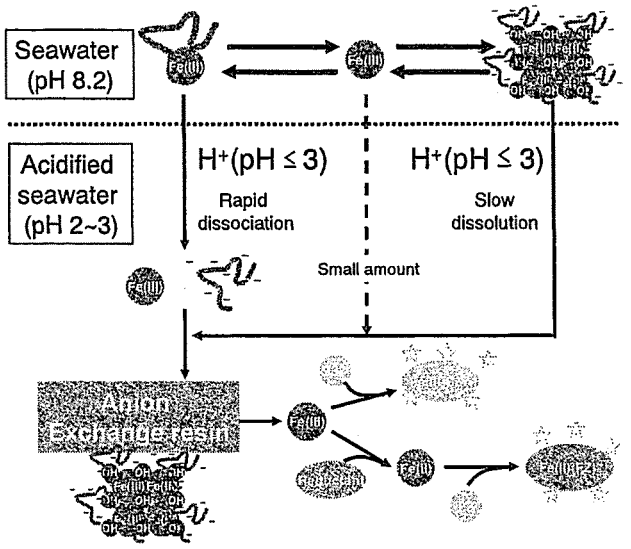

Figure 3. Conceptual model of the ion exchange separation method for organically complexed iron and ligand adsorbed amorphous ferric oxyhydroxide (AFO-L) in coastal water.

4. Production of Fe(II) via superoxide-mediated reduction of Fe(III)-organic complex in coastal water

In this work, the mechanism of SO-mediated reduction of Fe(III)L has been investigated (Figure 4). Since the formation of $Fe(II)(FZ)_3$ accompanied by the Fe(III)L reduction occur via non-dissociative and/or dissociative reduction pathways, the rate constant of reactions involved in the process (i.e. dissociation, complexation and redox reactions of ferrous and ferric iron in both organic and inorganic forms) was experimentally determined by using spectrophotometry and chemiluminescence-based method (Table 1).

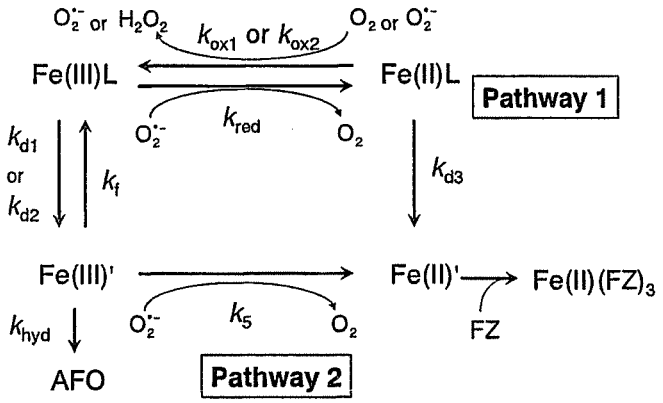

Figure 4. Conceptual model for superoxide-mediated reduction of organically complexed Fe(III) in the presence of ferrozine (FZ). Fe(III)L, Fe(II)L, Fe(II)', Fe(III)', AFO and $Fe(II)(FZ)_3$ indicate the organically complexed ferric and ferrous iron, dissolved inorganic species of ferrous and ferric iron, amorphous ferric oxyhydroxide and ferrous-ferrozine complex, respectively.

The results have shown that the ability of pico to nanomolar concentration of superoxide can reduce Fe(III) species and suggested the possibility of superoxide-mediated formation of bioavailable Fe(II) species in typical marine and coastal waters. The rates of Fe(II) production and the pathway are highly dependent on the characteristics of organic ligand and concentrations of superoxide and excess organic ligands. Under our experimental conditions, the second pathway in which Fe(II)' is formed by the reduction of liberated Fe(III)', is predominant for EDTA and citrate complexes, whereas the first pathway, where Fe(II)' is released after the direct

Table 1. The rate constants for the kinetics of iron and various organic ligands in seawater at pH 8.2.

Name	k_f ($\times 10^6$ M^{-1} s^{-1})	k_{d1} ($\times 10^{-4}$ s^{-1})	k_{d2} ($\times 10^{-5}$ s^{-1})	k_{d3} ($\times 10^{-4}$ s^{-1})	k_{d4} ($\times 10^{-5}$ s^{-1})	k_{ox1} ($M^{-1}s^{-1}$)	k_{ox2} ($\times 10^6$ $M^{-1}s^{-1}$)	k_{red} ($\times 10^4$ $M^{-1}s^{-1}$)	k_{NOM} ($L\,g^{-1}\,s^{-1}$)
DFA	3.6 (± 0.3)	14.7 (± 10.2)	2.5 (± 1.3)	13.4 (± 2.0)	7.0 (± 0.9)	19.2 (± 6.4)	10.5 (± 0.4)	2.9 (± 0.8)	6.7 (± 2.3)
AHS1	3.2 (± 0.9)	6.9 (± 2.0)	1.6 (± 1.1)	11.7 (± 1.1)	10.0 (± 1.8)	19.1 (± 3.3)	10.0 (± 0.5)	3.9 (± 0.4)	5.6 (± 0.8)
AHS2	2.6 (± 0.9)	7.4 (± 4.1)	2.1 (± 1.0)	12.3 (± 2.3)	11.1 (± 1.8)	12.4 (± 0.8)	8.7 (± 0.7)	5.6 (± 1.4)	4.3 (± 1.0)
AHS3	3.5 (± 0.1)	10.5 (± 2.9)	2.2 (± 0.9)	9.0 (± 2.5)	72.9 (± 20.6)	45.2 (± 3.6)	16.2 (± 0.8)	3.8 (± 0.6)	4.6 (± 0.8)
SFA1	7.3 (± 0.3)	11.1 (± 1.4)	4.2 (± 1.3)	19.5 (± 6.5)	10.9 (± 2.0)	25.4 (± 1.6)	14.6 (± 0.3)	3.8 (± 0.8)	13.7 (± 0.4)
SFA2	5.7 (± 0.3)	12.5 (± 2.6)	8.1 (± 2.6)	14.0 (± 2.2)	6.0 (± 0.7)	22.9 (± 7.9)	12.4 (± 0.6)	3.4 (± 0.6)	11.6 (± 0.6)
SFA3	5.0 (± 0.2)	12.0 (± 1.1)	3.5 (± 1.4)	14.7 (± 1.7)	6.3 (± 0.1)	25.3 (± 6.7)	11.4 (± 0.1)	2.0 (± 0.03)	7.0 (± 0.9)
SFA4	3.7 (± 0.6)	20.0 (± 11.0)	8.1 (± 2.9)	12.8 (± 1.5)	6.3 (± 0.6)	14.5 (± 4.3)	8.3 (± 0.2)	2.5 (± 0.7)	7.3 (± 0.4)
SFA5	6.1 (± 1.3)	6.9 (± 1.8)	8.3 (± 1.5)	12.7 (± 1.8)	6.4 (± 1.9)	52.0 (± 5.3)	23.0 (± 0.9)	3.9 (± 0.4)	13.1 (± 1.1)
SFA6	2.7 (± 0.2)	9.0 (± 4.5)	6.8 (± 2.8)	13.5 (± 1.3)	7.8 (± 0.9)	19.5 (± 1.6)	10.7 (± 0.6)	2.6 (± 0.7)	9.9 (± 3.3)
SFA7	6.2 (± 0.7)	15.4 (± 11.1)	6.8 (± 2.7)	12.5 (± 2.1)	8.1 (± 1.6)	21.3 (± 3.5)	11.6 (± 0.01)	3.0 (± 0.1)	10.5 (± 0.6)
SFA8	3.4 (± 0.7)	12.8 (± 3.2)	5.1 (± 2.6)	15.4 (± 1.0)	7.3 (± 0.3)	5.6 (± 2.6)	6.9 (± 1.8)	2.8 (± 0.1)	10.2 (± 7.6)
Cit	0.086 (± 0.04)	7.0 (± 3.8)			5.6 (± 0.5)	3.6 (± 0.1)	4.3 (± 0.4)	1.8 (± 0.6)	N.D.
EDTA	0.21 (± 0.01)		3.6 (± 1.5)		0.63 (± 0.3)	12.2 (± 6.4)	8.2 (± 0.1)	21.8 (± 1.1)	N.D.

reduction of the organic Fe(III) complex, is significant for the strong-binding ligand complexes of NOM (Figure 5). In natural coastal water containing NOM, therefore, Fe(II)' production via pathway 1 seems to be important. Because Fe(II)' production via each pathway is largely affected by the reactive oxygen species and competing strong iron chelators, the investigation of the pathway will provide significant information on the iron availability for uptake. These results suggest that both pathways have to be involved in the iron uptake model.

More than 99 % of dissolved iron in seawater is bound to an excess of strong organic ligands resulting in limitted concentration (< ~0.07 pM) of inorganic dissolved iron. Although the organic complexes react slowly with transport ligand at the cell surface and are not biologically permeable, there are accumulated evidences that oceanic microorganisms possess the ability to access to the strong-binding complexes. The iron bound to other siderophore and in-situ natural organic ligands are recently found to be available for uptake by oceanic phytoplankton in subantarctic waters. In addition, it has been documented that the biological and photochemical production of superoxide is prevalent in marine system. These evidences coupled with findings of the present study showing the ability of pico to nanomoar concentration of superoxide to significantly produce Fe(II) species suggests that superoxide-mediated reduction of organically complexed ferric iron may play a significant role in the iron uptake by coastal and marine microorganisms.

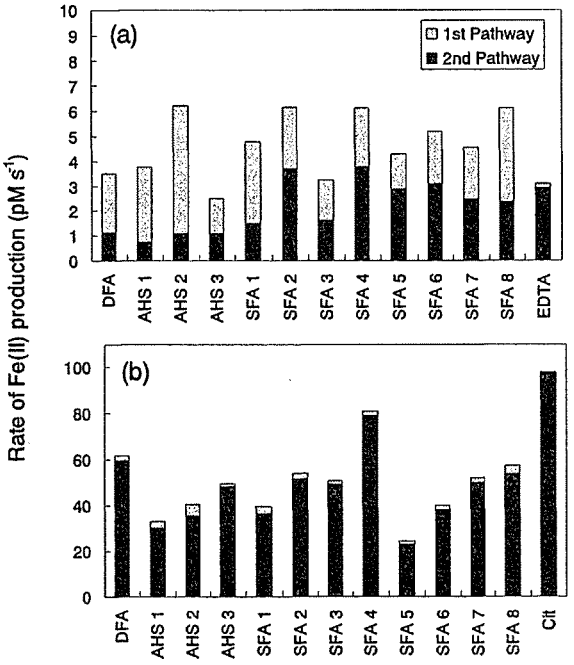

Figure 5. Predicted rate of Fe(II)' production for complexes by (a) strong-binding ligands and (b) weak-binding ligand of NOM. The model was calculated in a seawater containing 100 nM Fe(III) ligand complex, 1 mM FZ, 50 μM xanthine and 1 unit L^{-1} XO. In the calculation for SOL complexes, 200 nM Fe(III) and 10 μM ligands were used.

論文審査結果の要旨

本論文は，スーパーオキシドによる有機鉄および水酸化鉄の還元機構を，反応速度論的視点から明らかにしており，沿岸域における海洋性藻類の鉄摂取機構の解明へ向けて，有用な知見を示した。

第１章では，研究の背景として，沿岸域における鉄の形態および海洋性藻類の鉄摂取機構に関する既往の研究を整理している。具体的には，沿岸域の鉄の多くは，酸化状態である第二鉄形態(主に有機鉄と水酸化鉄)で存在するが，第一鉄(Fe(II))が最も海洋性藻類にとって摂取しやすい鉄の形態であること，また，多くの藍藻類はスーパーオキシドを生成し，生成されたスーパーオキシドは第二鉄種をFe(II)に還元する作用を持つことを示している。これらの知見から，海洋性藻類の鉄摂取機構にはスーパーオキシドが大きく関与し，鉄摂取機構の解明には沿岸域で卓越する水酸化鉄と有機鉄の還元動力学を詳細に調べることが必要不可欠であることを指摘している。

第２章では，沿岸域で優先する鉄の一種である水酸化鉄に着目し，スーパーオキシドによって生物利用可能なFe(II)へ還元される経路の解明を行っている。比色分析法を用いたFe(II)生成実験では，水酸化鉄の形成時間が増加するに伴い，酸化による水酸化鉄の化学的反応性が低下するため，Fe(II)生成速度も低下することを示した。さらに，反応速度論からFe(II)生成経路を予測した結果，水酸化鉄が解離してFe(II)が生成される経路が卓越することが明らかとなった。

第３章では，沿岸域において有機鉄と水酸化鉄を分離する手法を開発している。溶液のpHを低下させると，有機鉄のみが解離する。有機リガンドと水酸化鉄は負に帯電し，一方，有機リガンドから解離した鉄は正に帯電している。この性質を利用し，陰イオン交換樹脂により水酸化鉄と有機鉄の分離を試みた結果，陰イオン交換樹脂はこれらの分離に有効であることが示された。本分離手法は，化学的性質の異なる鉄種の分離を可能とすることから，沿岸域での鉄形態や生物利用性の研究において，その実用性は高いと考えられる。

第４章および第５章では，有機鉄の錯態形成・解離，酸化・還元反応に関して，それらの反応速度定数を実験的に算出した。これら反応速度定数の決定は，スーパーオキシドによる有機鉄の還元経路を解明するために必要不可欠である。本章における成果に関して特記すべきところは，従来の研究では濾過法等による物理的な鉄の分類では反応速度定数を正確に評価することができなかったが，本章では競合リガンド法や新規のケミルミネッセンス法を利用した実験系において，鉄の反応速度定数を決定することに成功したことである。

第６章では，４及び５章で得られた反応速度定数を用いて，スーパーオキシドによる有機鉄の還元動力学のモデル化および還元経路の解明を行った。この章では，まずFe(II)に関与する反応式から構成されるFe(II)生成モデルを構築し，このモデルを用いて実験的に算出されたFe(II)生成速度を説明することで，モデルの有用性を確認した。また，モデル計算から，鉄との結合強度が強いリガンドほど，有機鉄がスーパーオキシドにより直接還元され，その錯体が解離することでFe(II)が生成される経路が優位になることが明らかとなった。

第７章は結論である。

以上要するに本論文は，沿岸域に卓越する鉄種である有機鉄と水酸化鉄に関して，それらがスーパーオキシドにより還元される機構を明らかにし，さらに，生物利用可能なFe(II)の生成速度を定量的に評価することを可能とした。従って，その成果は，環境工学の発展に寄与するところが少なくない。

よって，本論文は博士(工学)の学位論文として合格と認める。